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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : G01N 23/00 — A1

(11) International Publication Number: WO 00/37927

(43) International Publication Date: 29 June 2000 (29.06.00)

(21) International Application Number: PCT/US99/30156

(22) International Filing Date: 16 December 1999 (16.12.99)

(30) Priority Data:
09/216,787 21 December 1998 (21.12.98) US

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(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: HIGH ENERGY PHOTOTHERAPEUTIC AGENTS

(57) Abstract

A high energy phototherapeutic agents or radiosensitizer agent comprised of a halogenated xanthene, or an agent that exhibits a preference for concentration in biologically sensitive structures in diseased tissue, and methods of treating and imaging using radiosensitizer agents in diseased tissue.

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HIGH ENERGY PHOTOTHERAPEUTIC AGENTS

BACKGROUND OF THE INVENTION

The present invention is directed to high energy phototherapeutic agents, or specifically to radiosensitizing and methods of treating and imaging using such phototherapeutic or radiosensitizer agents. More specifically, the treating and imaging is of diseased tissue, such as tumors, particularly cancerous tumors.

Diseased tissue or tumors, such as those for cancer, are often treated using ionizing radiation, in a process known as radiation therapy.

Radiation therapy (which typically uses electromagnetic radiation with energies of 1 keV or higher) for cancer typically works by attacking rapidly growing cells with highly penetrating ionizing radiation. Use of such radiation is attractive due to its ability to penetrate deeply into tissue, especially when diseased tissue is, or is located within, bone or other dense or opaque structures. Unfortunately, using rapid growth as the sole targeting criterion does not limit the effects of such treatment to cancer cells.

As a result, improvements have been made in the methods for delivery of the ionizing radiation to the site of the cancerous tumor so as to limit the effects of such radiation to the general area of the cancerous tumor. However, since healthy tissue and cancerous tissue typically have a similar biological response to radiation, a need exists to improve the potency of (or biological response to) the delivered radiation within and in the vicinity of the tumor, while not affecting the surrounding healthy tissue.

As an alternative to the use of ionizing radiation, photodynamic therapy (PDT) has been developed and shows considerable promise for treatment of a variety of cancers. Photodynamic therapy is the combination of a photosensitive agent with site-specific illumination (using non-ionizing, optical radiation) to produce a therapeutic response in diseased tissue, such as a tumor. In PDT, a preferential concentration of photosensitizer is to be located in the diseased tissue, either through natural processes or via localized application, and not in the healthy surrounding tissue. This provides an additional level of tissue specificity relative to that achievable through standard radiation therapy since PDT is effective only when a photosensitizer is present in tissue. As a result, damage to surrounding, healthy tissue can be avoided by controlling the distribution of agent. Unfortunately, when using conventional methods for the illumination step in PDT (1) the light required for such treatment is unable to penetrate deeply into tissue, and (2) the

physician has minimal spatial control of the treatment site. This is particularly troublesome whenever the diseased tissue or tumor is deeply seated or located within bone or other opaque structures. Some of the inventors of the present invention have been able to resolve many of these problems for PDT, as shown in commonly-assigned U.S. Patent No. 5,829,448.

Others, however, have focused their efforts on developing agents that are sensitized or activated by the ionizing radiation mentioned above. Potentially, the use of such radiation would enable treatment of more deeply seated diseased tissue than that possible with optical radiation. The agents used with such radiation are known as radiosensitizers. It is also desirable to achieve preferential concentration of the radiosensitizer in the diseased tissue, either through natural processes or via localized application, so as to provide additional specificity relative to that achievable through standard radiation therapy. The desired result is for radiation to become more efficacious when the radiosensitizer is present in tissue, so that less radiation is needed to treat the lesion tumor or other diseased tissue, and accordingly, potential damage to surrounding healthy tissue, resulting from collateral exposure to the radiation, is reduced. Hence, safety and efficacy would then be improved.

The ultimate success or failure of the radiosensitizer approach depends on: (1) therapeutic performance of agents, and (2) disease specificity in the site of activation. Currently used agents and targeting approaches, however, have had unacceptable results in each of these categories.

The therapeutic performance of a radiosensitizer is primarily a function of enhanced absorption of the applied radiation dose in sensitized tissues relative to that in non-sensitized tissues. This differential absorption is commonly effected by use of agents having a high absorption cross-section for a particular type of radiation (such as x-rays). For example, metal or halogen atoms are often used, either in atomic form or incorporated into a molecular carrier, due to their high x-ray cross-section. Absorption of x-rays by such atoms appears to lead to secondary radiative emissions, ionization, and other chemical or physical processes that increase the localized cytotoxicity of the applied energy (i.e., radiation-induced cell death, or "light cytotoxicity").

However, a high light cytotoxicity is not enough to make an agent an acceptable agent. The agents must also have a negligible effect when energy is not applied (i.e., have a low toxicity in the absence of radiation, or "dark cytotoxicity"). Unfortunately, many

agents presently under investigation as radiosensitizers have the disadvantage of either: (a) a relatively high dark cytotoxicity or (b) a low light (cytotoxicity)-to-dark cytotoxicity ratio which limits their effectiveness and acceptability. Agents having a high light-to-dark cytotoxicity ratio are desirable because they (1) can be safely used over a range of dosages, (2) will exhibit improved efficacy at the treatment site (due to the compatibility with use at higher dosages as a consequence of their relative safety), and (3) will be better tolerated throughout the patient's body.

An additional problem with many current radiosensitizers is that the agent does not achieve significant preferential concentration in tumors. Specifically, most radiosensitizer targeting has been based on physical targeting, such as diffusion into tumors through leaky neurovasculature, which ultimately succeed or fail based on permeability of the tumor to agents that are aqueously soluble or are in a suspension formulation. As a result, large doses of the agent typically need to be administered, either locally or systemically, so as to saturate all tissues, hopefully reaching a therapeutic level in the desired treatment region or target. After such agent administration, a patient has to wait a clearance time of hours to days to allow excess agent to hopefully clear from healthy living tissues surrounding the desired treatment site. Thereafter, irradiation of residual agent at the treatment site hopefully produces the desired cytotoxic effect in the diseased tissue. This approach may unfortunately also damage healthy surrounding tissue by undesired but unavoidable activation of residual agent still present in the healthy surrounding tissue. One approach to solving this problem is to couple the radiosensitizer with a moiety capable of providing improved biotargetting of the diseased tissue. This, however, has proven to be very difficult to achieve.

It would also be highly desirable if the radiosensitizer could be used to improve identification of target size, location and depth so that the therapeutic radiation could be more precisely delivered to the target, such as a cancerous tumor. Combined diagnostic use (as a contrast agent) and therapeutic use (as a radiosensitizer) of the agent would reduce risk to the patient by (1) reducing the number of required procedures necessary for diagnosis and treatment, (2) reducing the overall diagnosis and treatment time, and (3) reducing cost.

Accordingly, one object of the present invention is to develop new radiosensitizers that have one or more of the following characteristics: (1) improved light-to-dark cytotoxicity ratio; (2) improved accumulation of agent into diseased tissue with strong

contrast between diseased and healthy tissue; (3) rapid clearance from normal tissue; and (4) capability of combined imaging and therapy. Further desirable characteristics include low agent cost, and significant regulatory history (so as to facilitate acceptance by the regulatory and medical communities).

SUMMARY OF THE INVENTION

The present invention is directed to a radiosensitizer agent for treatment of diseased tissue using radiosensitization or ionizing radiation comprising a halogenated xanthene. Preferably, the halogenated xanthene is Rose Bengal or its derivative.

In a further embodiment of the present invention, the radiosensitizer agent also acts as an imaging contrast agent.

The present invention is also directed to a radiosensitizer agent for treatment of diseased tissue using radiosensitization or ionizing radiation wherein the agent exhibits a preference for concentration in biologically sensitive structures in tissue, such as, for example, cellular membranes. Preferably, the agent biologically or chemically targets the biologically sensitive structures.

Further, the present invention is directed to a method for treating diseased tissue.

One embodiment of the method of the present invention includes the steps of administering a radiosensitizer agent, preferably a halogenated xanthene, a portion of radiosensitizer agent being retained in diseased tissue; and treating the diseased tissue with x-rays or other ionizing radiation to activate the radiosensitizer agent in the diseased tissue.

A further embodiment of the method of the present invention includes the step of imaging a patient using the radiosensitizer agent to identify the diseased tissue.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1a is an illustration of the chemical structure of Rose Bengal;

FIGURE 1b is an illustration of the chemical structure of a halogenated xanthene;

FIGURE 2 illustrates the CAT scan image of test tubes of Rose Bengal, standard x-ray contrast agents and a control;

FIGURE 3 illustrates a CAT scan of a range of concentrations of the solutions of Figure 3;

FIGURE 4 is a graph of energy versus x-ray cross-section for halogens.

DETAILED DESCRIPTION OF THE PRESENTLY PREFERRED EMBODIMENTS

The present invention is directed to agents that can efficiently interact with x-rays or other types of ionizing radiation to produce a beneficial biological response and to methods of treatment and imaging using such agents.

The inventors of the present invention have discovered that radio dense agents, such as the halogenated xanthenes discussed *infra*, which exhibit a preference for concentration in cellular membranes and other key components and structures of diseased tissue, will exhibit additional therapeutic dose enhancement over that possible with previously known agents or enhancement mechanisms. This additional dose enhancement is a consequence of increased radiosensitization yield of such agents owing to improved proximity of such agents, upon interaction with diseased tissue, to sensitive structures during irradiation and subsequent radiosensitization. Specifically, most radiosensitizers function by absorbing highly-penetrating energy (which in itself has little direct interaction with tissue), and then releasing this energy in a less-penetrating, more cytotoxic form (such as lower-energy re-emission) that is capable of interacting primarily only with proximal, biologically-sensitive structures or materials (such as cellular membranes and genetic material).

Thus, any radiodense agent, such as halogenated xanthenes, that exhibits chemical or biological targeting to such biologically-sensitive structures or materials, and which thereby becomes substantially concentrated in areas in physical proximity to such structures or materials, will increase the overall efficiency of radiosensitization (i.e. conversion of high-energy stimulating excitation into localized cytotoxic effects). This yield enhancement results from the increased probability that proximally-released energy will interact favorably with the sensitive target (before annihilating or otherwise dissipating in an inefficacious manner) whenever the agent responsible for such re-emission is concentrated as close as possible to such a target. Stated in simple terms, the released energy, having a short mean free path, will have a higher probability of interacting with the target if it is emitted from an agent located closer to the target.

Such approaches to radiosensitization enhancement are not taught in the prior art, which are based primarily on permeability-based targeting. In contrast, targeting as taught by the present invention uses the superior approach based on chemical or biological targeting. This type of targeting can be effected by chemical partitioning of the agent at,

near or into the target (for example, using an agent that partitions into cell walls, such as Rose Bengal discussed *infra*, the chemical structure of which is illustrated in Figure 1a), by controlled agent delivery at, near or into the target (for example by encapsulation of an agent, such as Rose Bengal, into a delivery vehicle, such as a micelle, nanoparticle, or liposome, that interacts preferentially with a target site, such as cell walls, and may adhere, fuse, combine, or otherwise interact in such a way that agent is delivered to the target), or by physically increasing local concentration of agent at, near or into the target, for example by localized delivery via injection, flooding, or spraying.

Preferably, these agents have a large x-ray cross-section, a high light-to-dark cytotoxicity ratio, a preference for accumulation in diseased tissue, low agent cost, rapid clearance from normal tissue, and a significant regulatory history (so as to facilitate acceptance by the regulatory and medical communities).

Applicants have discovered a class of agents that fits this criteria and is preferably used in the present invention. These agents are referred to as halogenated xanthenes and are illustrated in Figure 1b, where the symbols X, Y, and Z represent various elements present at the designated positions, and the symbols R^1 and R^2 represent various functionalities present at the designated positions. Chemical and physical properties (such as the chemical constituents at positions X, Y, and Z and the functionalities R^1 and R^2, along with molecular weight) of representative halogenated xanthenes are summarized in attached Table 1. While many of the halogenated xanthenes are highly soluble in aqueous solution, in general all demonstrate a preference for selective partitioning into hydrophobic environments, such as within cell membranes.

In general, halogenated xanthenes are characterized by a low dark cytotoxicity and chemical properties that are substantially unaffected by the local chemical environment or the attachment of functional derivatives at positions R^1 and R^2. Moreover, the halogenated xanthenes will target some tumors or other diseased tissues based on their inherent selective partitioning properties.

A specific example of a halogenated xanthene is Rose Bengal (4,5,6,7-tetrachloro-2',4',5',7'-tetraiodofluorescein; see 10 in Figure 1a). In particular, Rose Bengal has been found to accumulate preferentially in (i.e. target) some tumors and other diseased tissues. Moreover, Rose Bengal has other desirable characteristics such as a negligible dark cytotoxicity, relatively low cost, the ability to clear rapidly from the body, and a partially established regulatory history. Furthermore, the inventors have found that the special

chemical properties of Rose Bengal allow it to be dissolved in aqueous solution at high concentrations while retaining a significant preference for hydrophobic environments, such as within cell membranes.

The present inventors have also discovered that the facility with which the halogenated xanthenes target specific tissues or other sites can be optimized by attachment of specific functional derivatives at positions R^1 and R^2, so as to change the chemical partitioning or biological activity of the agent. For example, attachment of one targeting moiety or more at positions R^1 or R^2 can be used to improve targeting to specific tissues, such as cancerous tumor tissues or sites of localized infection. These targeting moieties include DNA, RNA, amino acids, proteins, antibodies, ligands, haptens, carbohydrate receptors or complexing agents, lipid receptors or complexing agents, protein receptors or complexing agents, chelators, encapsulating vehicles, short- or long-chain aliphatic or aromatic hydrocarbons, including those containing aldehydes, ketones, alcohols, esters, amides, amines, nitriles, azides, or other hydrophilic or hydrophobic moieties.

An example of this feature would be to combine Rose Bengal with a lipid (at position R^1, via esterification, so as to increase the lipophilicity of Rose Bengal, and thereby modify its targeting properties in a patient. Such a modified agent could be administered directly as a micelle suspension, or delivered in conjunction with a delivery vehicle, such as a surfactant, and would exhibit increased targeting to tumor cells. Suitable formulations of such an agent include topical creams and lotions, and liquids for intravenous or parenteral injection.

Figure 4 demonstrates that strong absorption for the halogens of the halogenated xanthenes occurs well below the energies used for standard diagnostic or therapeutic x-ray devices, which generally use energies greater than 30 keV. In fact, the halogen content of the halogenated xanthenes makes this class of agent potent x-ray absorbers, and thus highly suitable as radiosensitizers. Further, since x-ray cross-section increases substantially in the order $F < Cl < Br < I$, it is preferred that those halogenated xanthenes with a large content of I or Br be used for x-ray sensitization. Furthermore, tests indicate that the presence of I or Br yields enhanced sensitization relative to that possible with other halogens. Therefore, as shown in Table 1, Tetrabromoerythrosin, Rose Bengal, Phloxine B, Erythrosin B, and Eosin Y have larger x-ray cross-sections than Solvent Red or Eosin B as a consequence of respective differences in halogen content, and thereby are preferred for use as x-ray sensitizing agents. More preferably, the high iodine

content of Rose Bengal and its derivatives and the additional bromine substitution of 4,5,6,7-Tetrabromoerythrosin and its derivatives, makes these agents the most preferred x-ray sensitizing agents of this class.

Accordingly, in a preferred embodiment of the present invention, at least one halogenated xanthene is used as an x-ray sensitizer or radiosensitizer agent for treatment of diseased tissue using radiosensitization. Prior to radiosensitization, the agent can be administered orally, systemically (e.g. by an injection), or topically, in a manner well known in the art. In a further preferred embodiment of the present invention, Rose Bengal or its derivatives or 4,5,6,7-Tetrabromoerythrosin or its derivatives is the radiosensitizer agent. It is also preferred that x-rays or other ionizing radiation with energy $\geq$ approximately 1 keV and $\leq$ 1000 MeV be used to activate the agent. Preferably, the agent is activated using x-rays having an energy in excess of 30 keV.

Applicants have also discovered that halogenated xanthenes can be used as an imaging contrast agent for x-ray or other ionizing radiation imaging, such as CAT scan, fluorography or other related procedures. In particular, the inventors have discovered that halogenated xanthenes are particularly proficient as imaging contrast agents because of their large x-ray cross-sections and because their chemical structure, which has a high electron density due to their significant halogen content, renders them opaque to x-rays or other ionizing radiation used for imaging. For example, Rose Bengal is highly opaque to the x-rays used for CAT scan or normal x-ray imaging. Figures 2 and 3 illustrate the opaqueness of Rose Bengal versus standard x-ray contrast agents and a control. These figures are drawings of actual pictures of experiments done by the inventors of the present invention. For example, the CAT scan image of test tubes containing various solutions shown in Figure 2 demonstrates that iodine (350 mgI/mL in aqueous base), Rose Bengal (225 mg halogen/mL in saline), and Omnipaque™ (350 mgI/mL Iohexol) have similar x-ray densities. Furthermore, these densities are dramatically greater than that of a control (saline). A CAT scan image of various dilutions of these same solutions (held in wells in a 96-well sample plate) illustrated in the drawing in Figure 3 further demonstrates that Rose Bengal shows comparable response to that of the standard x-ray contrast agents across a range of concentrations.

Accordingly, it is a further preferred embodiment of the present invention to use at least one halogenated xanthene agent as an imaging contrast agent for x-ray or

ionization radiation based imaging and detection of diseased tissue, and then treat the detected diseased tissue by radiosensitization of the residual agent present in such tissue.

This description has been offered for illustrative purposes only and is not intended to limit the invention of this application, which is defined in the claims below. For example, it will be clear to those of ordinary skill in the art that the targeting described herein for the specific example of the halogenated xanthenes can be adapted or otherwise applied to other radiodense materials, including conventional radiosensitizers.

Table I. Physical Properties of Example Halogenated Xanthenes:

Compound	Substitution					MW (g)
	X	Y	Z	R^1	R^2	
Fluorescein	H	H	H	Na	Na	376
4',5'-Dichlorofluorescein	Cl	H	H	Na	Na	445
2',7'-Dichlorofluorescein	H	Cl	H	Na	Na	445
4,5,6,7-Tetrachlorofluorescein	H	H	Cl	H	H	470
2',4',5',7'-Tetrachlorofluorescein	Cl	Cl	H	Na	Na	514
Dibromofluorescein	Br	H	H	Na	Na	534
Solvent Red 72	H	Br	H	H	H	490
Diiodofluorescein	I	H	H	Na	Na	628
Eosin B	NO_2	Br	H	Na	Na	624
Eosin Y	Br	Br	H	Na	Na	692
Ethyl Eosin	Br	Br	H	C_2H_5	K	714
Erythrosin B	I	I	H	Na	Na	880
Phloxine B	Br	Br	Cl	Na	Na	830
Rose Bengal	I	I	Cl	Na	Na	1018
4,5,6,7-Tetrabromoerythrosin	I	I	Br	Na	Na	1195

What is claimed as new and desired to be protected by Letters Patent is set forth in the appended claims.

We claim:

1. A radiosensitizer agent for treatment of diseased tissue using radiosensitization or ionizing radiation comprising: a halogenated xanthene.

2. The agent of Claim 1, wherein said halogenated xanthene is selected from the group comprising Rose Bengal and its derivatives.

3. The agent of Claim 1, wherein said halogenated xanthene is selected from the group comprising 4,5,6,7-Tetrabromoerythrosin and its derivatives.

4. The agent of Claim 1 wherein said halogenated xanthene includes as a functional derivative a targeting moiety selected from the group comprising DNA, RNA, amino acids, proteins, antibodies, ligands, haptens, carbohydrate receptors or complexing agents, lipid receptors or complexing agents, protein receptors or complexing agents, chelators, encapsulating vehicles short- or long-chain aliphatic or aromatic hydrocarbons, including those containing aldehydes, ketones, alcohols, esters, amides, amines, nitriles, azides, or other hydrophilic or hydrophobic moieties.

5. The agent of Claim 1 wherein said radiosensitizer agent also is an imaging contrast agent.

6. The agent of Claim 5 wherein said radiosensitizer acts as an imaging contrast agent for CAT scan.

7. The agent of Claim 5 wherein said radiosensitizer acts as an imaging contrast agent for X-ray imaging.

8. The agent of Claim 1 wherein said halogenated xanthene has a large content of an element selected from the group comprising iodine and bromine.

9. The agent of Claim 1 wherein said agent is a halogenated xanthene selected from the group comprising Phloxine B, Erythrosin B and Eosin Y and their derivatives.

10. The agent of Claim 1 wherein said halogenated xanthene is activated using x-rays having an energy greater than 30 keV.

11. The agent of Claim 1 wherein said agent is encapsulated in a delivery vehicle, said vehicle being selected from the group comprising a micelle, nanoparticle, and liposome.

12. A radiosensitizer agent for treatment of diseased tissue using radiosensitization or ionizing radiation wherein said agent exhibits a preference for concentration in biologically sensitive structures in tissue.

13. The agent of Claim 12 wherein said agent exhibits a preference for concentration in cellular membranes.

14. The agent of Claim 12 wherein said agent biologically targets said biologically sensitive structures.

15. The agent of Claim 12 wherein said agent chemically targets said biologically sensitive structures.

16. The agent of Claim 14 wherein said targeting is by chemical partitioning of the agent at a position at, near or into the biologically sensitive structure.

17. The agent of Claim 14 wherein said targeting is by controlling agent delivery at a position at, near or into the biologically sensitive structure.

18. The agent of Claim 17 wherein said agent is delivered by encapsulation of said agent in a delivery vehicle.

19. The agent of Claim 18 wherein said agent is Rose Bengal or its derivatives.

20. The agent of Claim 19 wherein said delivery vehicle is selected from the group comprising a micelle, a nanoparticle and a liposome.

21. The agent of Claim 14 wherein said targeting is by physically increasing local concentration of said agent at a position at, near or into the biologically sensitive structure.

22. The agent of Claim 21 wherein said physical increasing local concentration of said agent is selected from the group comprising injection, flooding and spraying.

23. The agent of Claim 15 wherein said targeting is by chemical partitioning of the agent at a position at, near or into the biologically sensitive structure.

24. The agent of Claim 15 wherein said targeting is by controlling agent delivery at a position at, near or into the biologically sensitive structure.

25. The agent of Claim 24 wherein said agent is delivered by encapsulation of said agent in a delivery vehicle.

26. The agent of Claim 25 wherein said agent is Rose Bengal.

27. The agent of Claim 26 wherein said delivery vehicle is selected from the group comprising a micelle, a nanoparticle and a liposome.

28. The agent of Claim 15 wherein said targeting is by physically increasing local concentration of said agent at a position at, near or into the biologically sensitive structure.

29. The agent of Claim 28 wherein said physical increasing local concentration of said agent is selected from the group comprising injection, flooding and spraying.

30. The agent of Claim 12, wherein said agent is a halogenated xanthene.

31. A method of treating diseased tissue comprising the steps of:

administering a radiosensitizer agent to a patient, a portion of said radiosensitizer agent being retained in said diseased tissue; and

treating said diseased tissue with x-rays or other ionizing radiation so as to activate said retained radiosensitizer agent in said diseased tissue,

wherein said radiosensitizer agent is a halogenated xanthene.

32. The method of Claim 31 wherein said halogenated xanthene is Rose Bengal or its derivatives.

33. The method of Claim 31 wherein said halogenated xanthene includes as a functional derivative a targeting moiety selected from the group comprising DNA, RNA, amino acids, proteins, antibodies, ligands, haptens, carbohydrate receptors or complexing agents, lipid receptors or complexing agents, protein receptors or complexing agents, chelators, encapsulating vehicles, short- or long-chain aliphatic or aromatic hydrocarbons, including those containing aldehydes, ketones, alcohols, esters, amides, amines, nitriles, azides, or other hydrophilic or hydrophobic moieties.

34. The method of Claim 31 further comprising the step of imaging said patient using said radiosensitizer agent and radiation to identify said diseased tissue.

35. The method of Claim 34 wherein imaging is accomplished through a method selected from the group comprising computerized axial tomography and x-ray imaging.

36. The method of Claim 31 wherein said halogenated xanthene is selected from the group comprising iodinated and brominated halogenated xanthenes.

37. The method of Claim 34 wherein said halogenated xanthene is selected from the group comprising Rose Bengal, Phloxine B, Erythrosin B and Eosin Y and their derivatives.

38. The method of Claim 31 wherein said agent is administered by localized delivery.

39. The method of Claim 31 wherein said agent is administered via injection

40. The method of Claim 31 wherein said agent is administered by flooding.

41. The method of Claim 31 wherein said agent is administered by spraying.

42. The method of Claim 31 wherein said agent is encapsulated in a delivery vehicle, said vehicle being selected from the group comprising a micelle, nanoparticle, and liposome.

43. The method of Claim 31 further comprising biologically targeting of biologically sensitive structures in said diseased tissue by said agent.

44. The method of Claim 31 further comprising chemically targeting of biologically sensitive structures in said diseased tissue by said agent.

45. The method of Claim 43 wherein said targeting is by chemical partitioning of the agent at a position at, near or into the biologically sensitive structure.

46. The method of Claim 43 wherein said targeting is by controlling agent delivery at a position at, near or into the biologically sensitive structure.

47. The method of Claim 43 wherein said biologically sensitive structure is cellular membranes in the diseased tissue.

48. The method of Claim 44 wherein said targeting is by chemical partitioning of the agent at a position at, near or into the biologically sensitive structure.

49. The method of Claim 44 wherein said targeting is by controlling agent delivery at a position at, near or into the biologically sensitive structure.

50. The method of Claim 44 wherein said biologically sensitive structure is cellular membranes in the diseased tissue.

51. The agent of Claim 1 wherein said ionizing radiation is approximately greater than or equal to 1 keV and less than or equal to approximately 1000 MeV.

52. The agent of Claim 12 wherein said ionizing radiation is approximately greater than or equal to 1 keV and less than or equal to approximately 1000 MeV.

53. The method of Claim 31 wherein said ionizing radiation is approximately greater than or equal to 1 keV and less than or equal to approximately 1000 MeV.

Fig. 1a

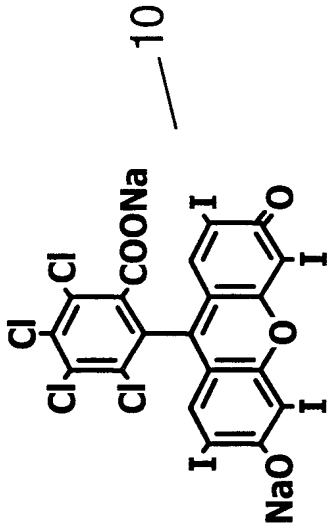
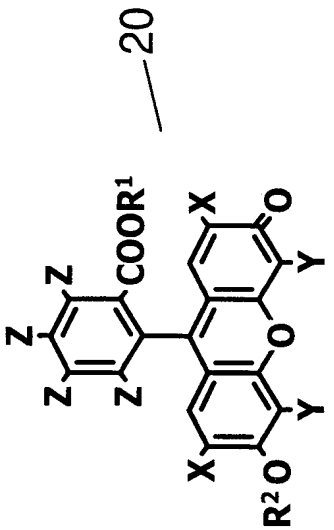


Fig. 1b



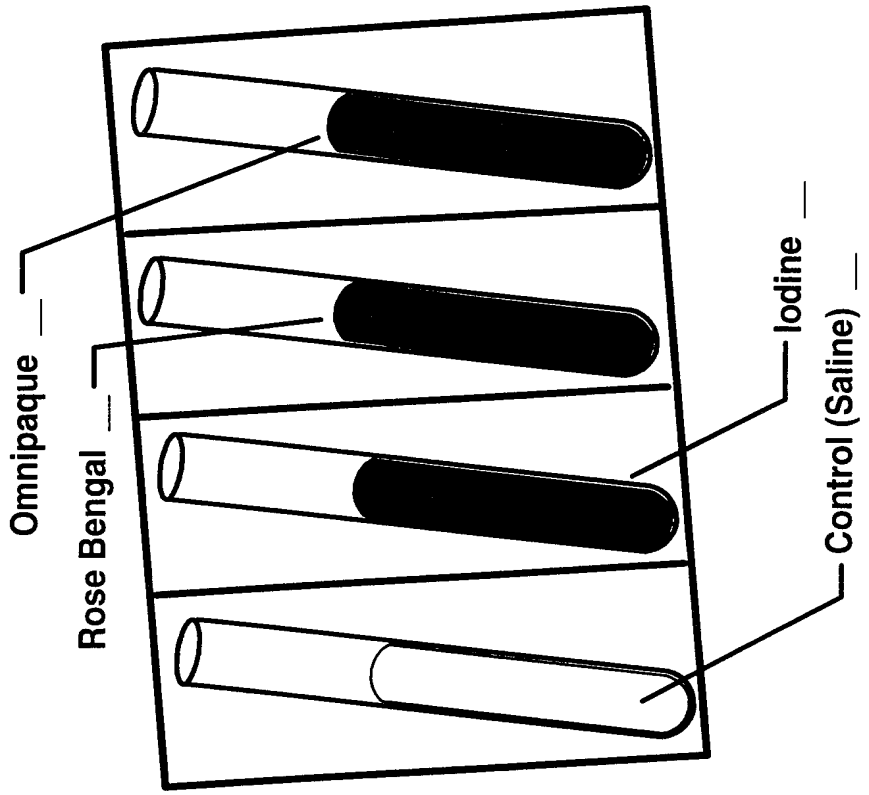


Fig. 2

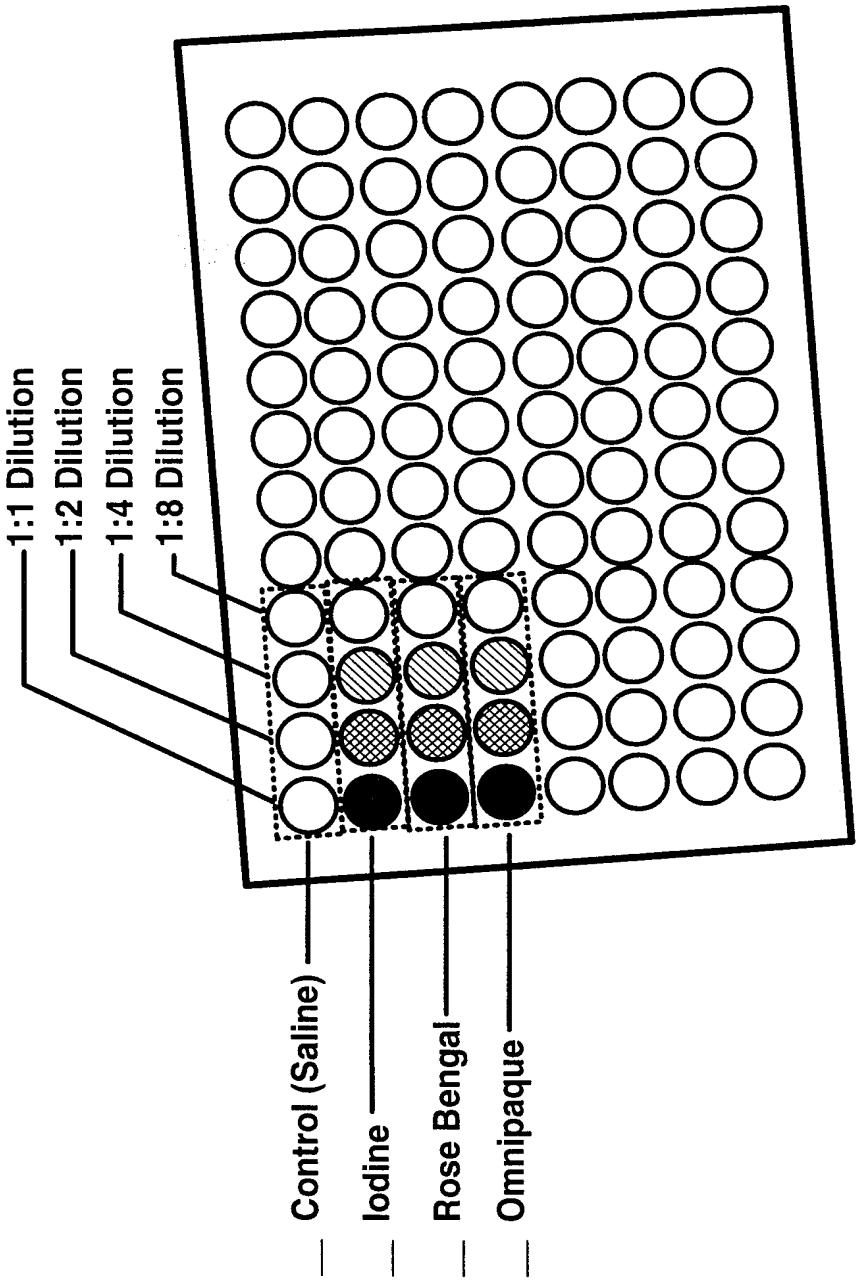

1:1 Dilution
1:2 Dilution
1:4 Dilution
1:8 Dilution
Control (Saline)
Iodine
Rose Bengal
Omnipaque

Fig. 3

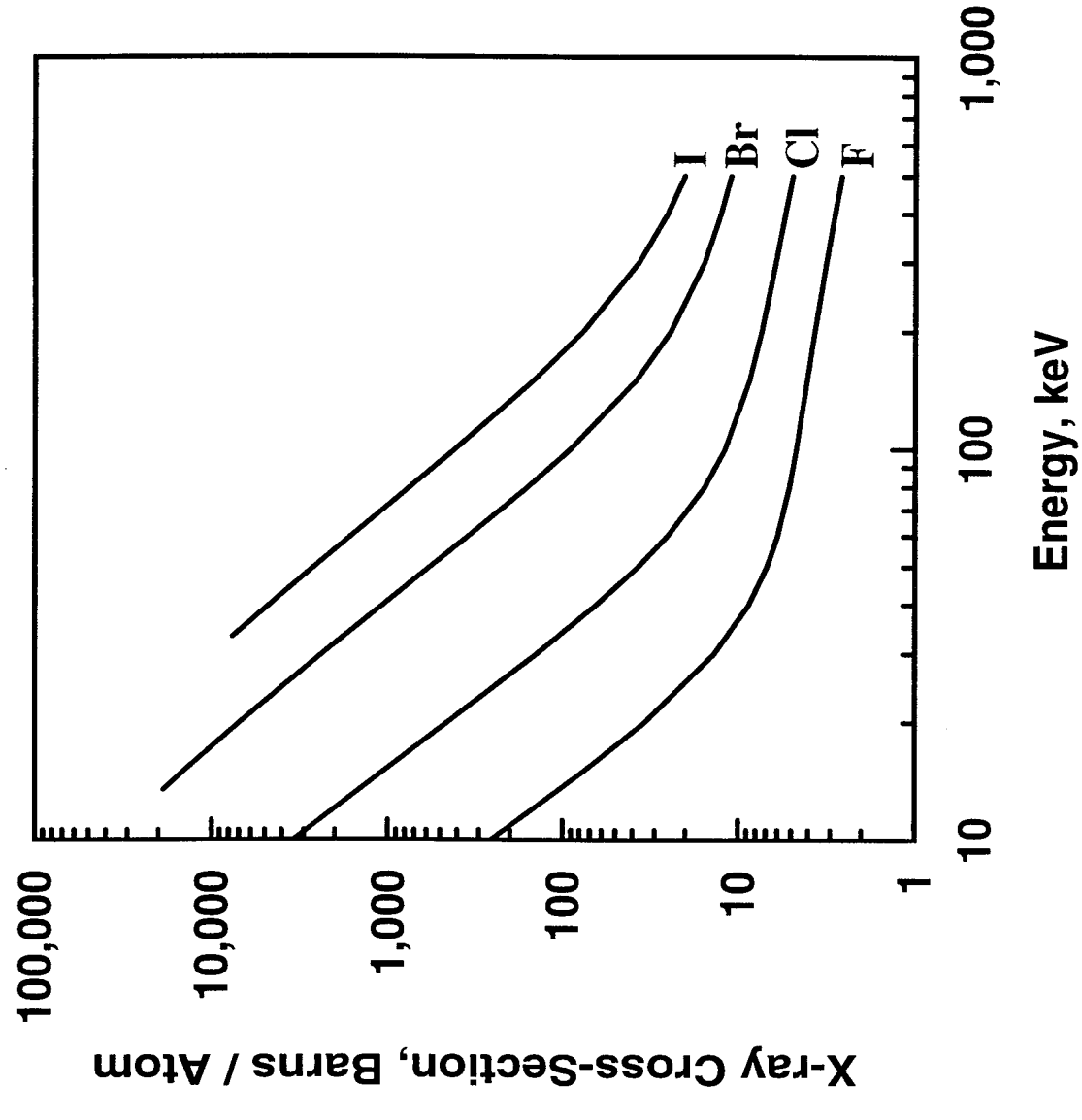

X-ray Cross-Section, Barns / Atom
100,000
10,000
1,000
100
10
1
Energy, keV
10
100
1,000
I
Br
Cl
F

Fig. 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/30156

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :G01N 23/00
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/450; 436/56, 57, 58, 63, 829; 128/66, 366, 371, 374; 128/898; 604/20; 607/2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, EMBASE, SCISEARCH, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SERAFINI et al., Iodine-123-Rose Bengal: An Improved Hepatobiliary Imaging Agent. Journal of Nuclear Medicine. January 1975. Vol 16. No. 7, pages 629-632.	1-3, 6-10, 31-32, 34-39, 44, 51, 53
Y	US 5,780,052 A (KHAW et al.) 14 July 1998, see col. 3-4, col. 7-8, col. 16-17.	1-53
Y	US 3,868,950 A (KATO) 04 March 1975, see col. 1-2.	1-3, 5-10, 12, 14-15, 21-22, 28-32, 34-38, 40-41, 44-45
A	NECKERS et al. Rose Bengal and Derivatives. Photochemistry and Photobiology A Chemistry Vol. 47. pages 1-29. 1989.	1-53

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 26 FEBRUARY 2000	Date of mailing of the international search report 08 MAR 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer GAILENE R. GABEL Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/30156

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,829,448 A (FISHER et al.) 03 November 1998. see Summar,page 3. col.1.	1-53

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/30156

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/450; 436/56, 57, 58, 63, 829; 128/66, 366, 371, 374; 128/898; 604/20; 607/2